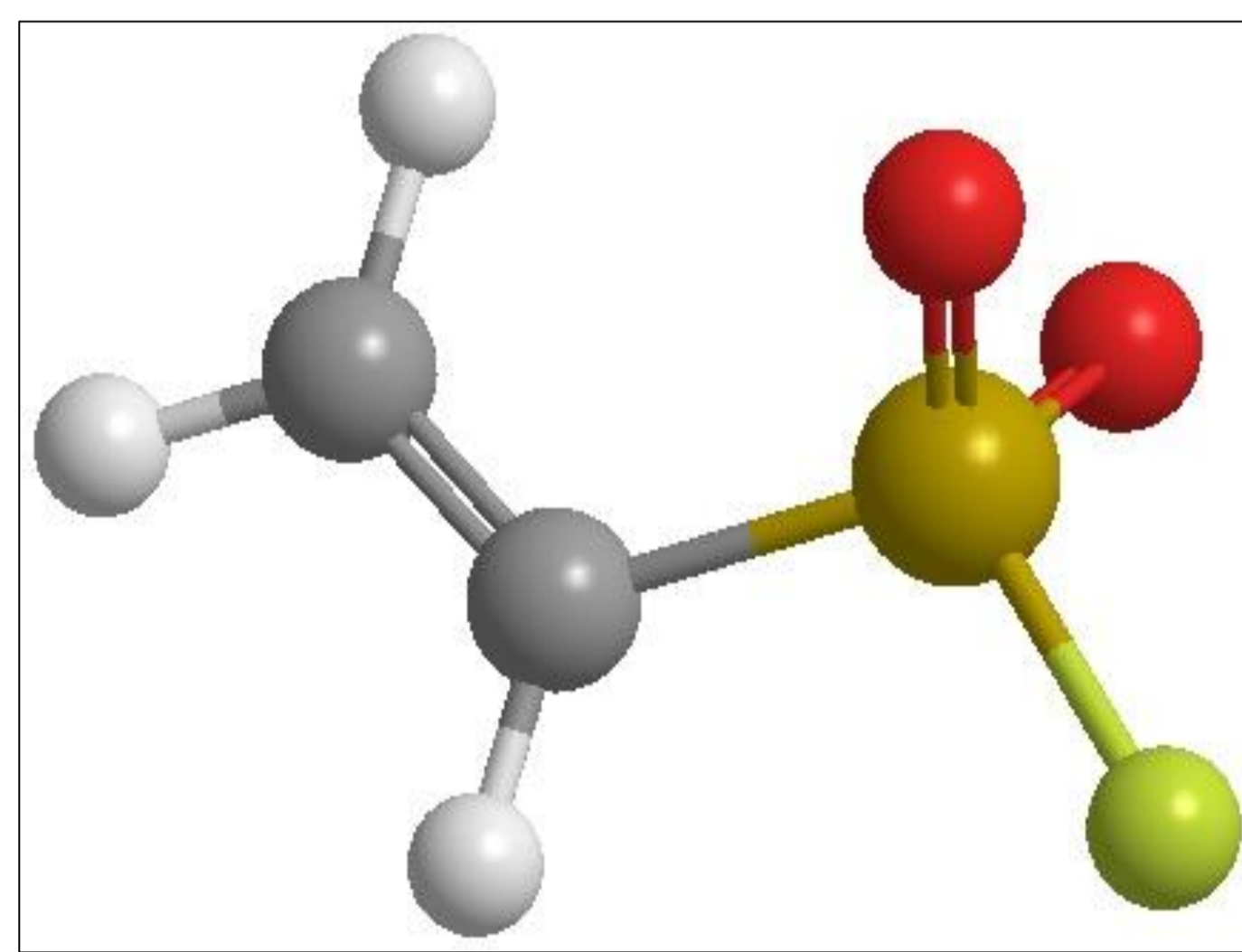


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INTRODUCTION

Ethenesulfonyl fluoride (ESF)¹



- Strong Michael acceptor that reacts with soft nucleophiles (–NH₂, –SH)
- Conjugation under mild conditions: fast kinetics at room temperature
- Water compatible: easy formulation
- Small, polar molecule: less chance to change the pharmacological properties of the biological vector (eg. target affinity, absorption, distribution and clearance)

Why [¹⁸F]ESF?

- PET Imaging: modality for observing physiological processes
- Sulfonyl fluorides: stable *in vivo* and *in vitro*, therapeutic use
- Ideal prosthetic group for radiolabelling proteins & peptides

This work

- Radiosynthesis of [¹⁸F]ESF
- Conjugation with model amino acids, peptides and proteins

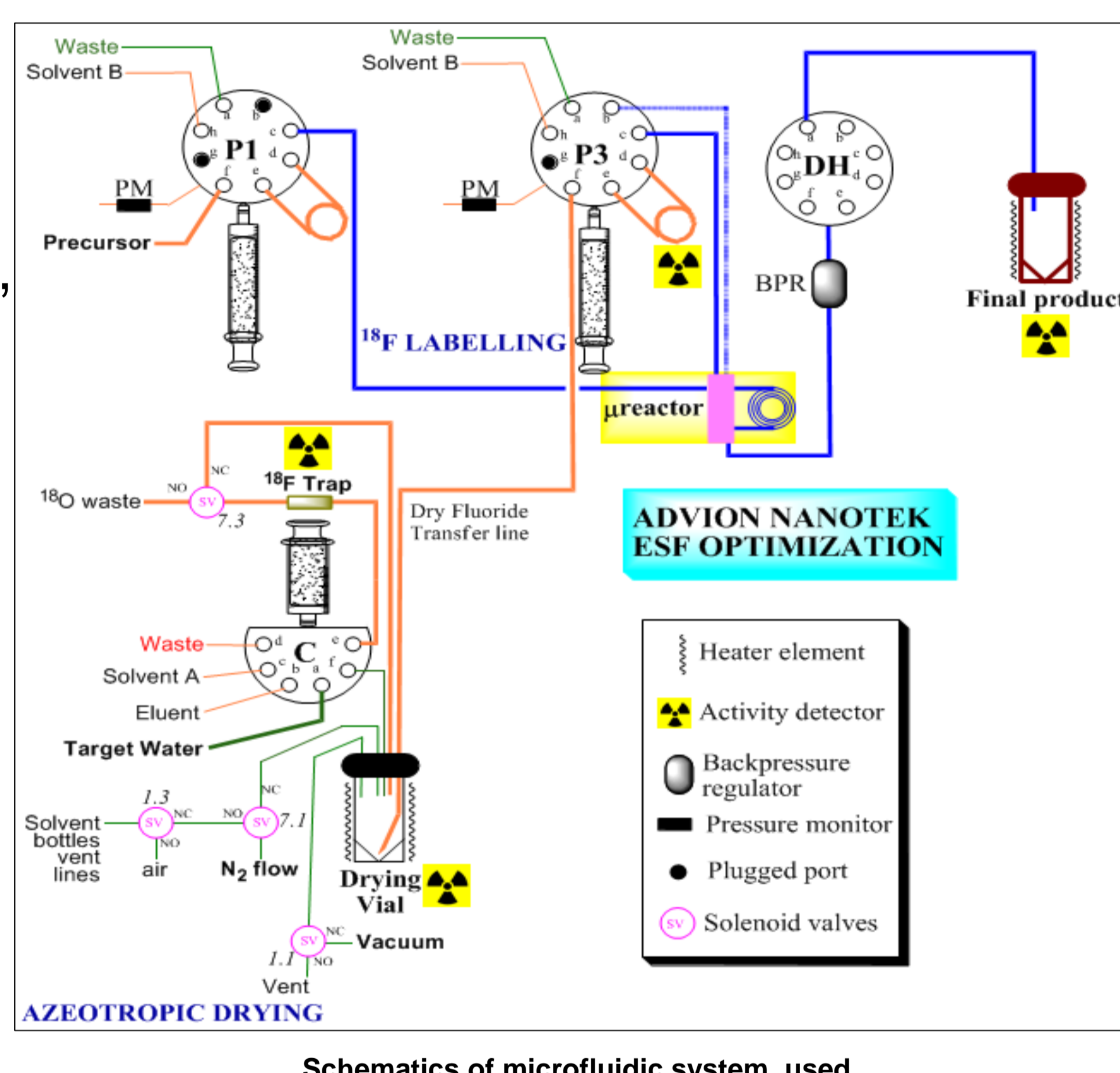
MATERIALS AND METHODS

Radiosynthesis of [¹⁸F]ESF

- Isotopic exchange on [¹⁹F]ESF
- Radiosynthesis performed on a microfluidic system (Advion NanoTek)² for the radioisotopic exchange reaction

- Evaluated different parameters including activator, solvent, reaction concentration, temperature and residence time

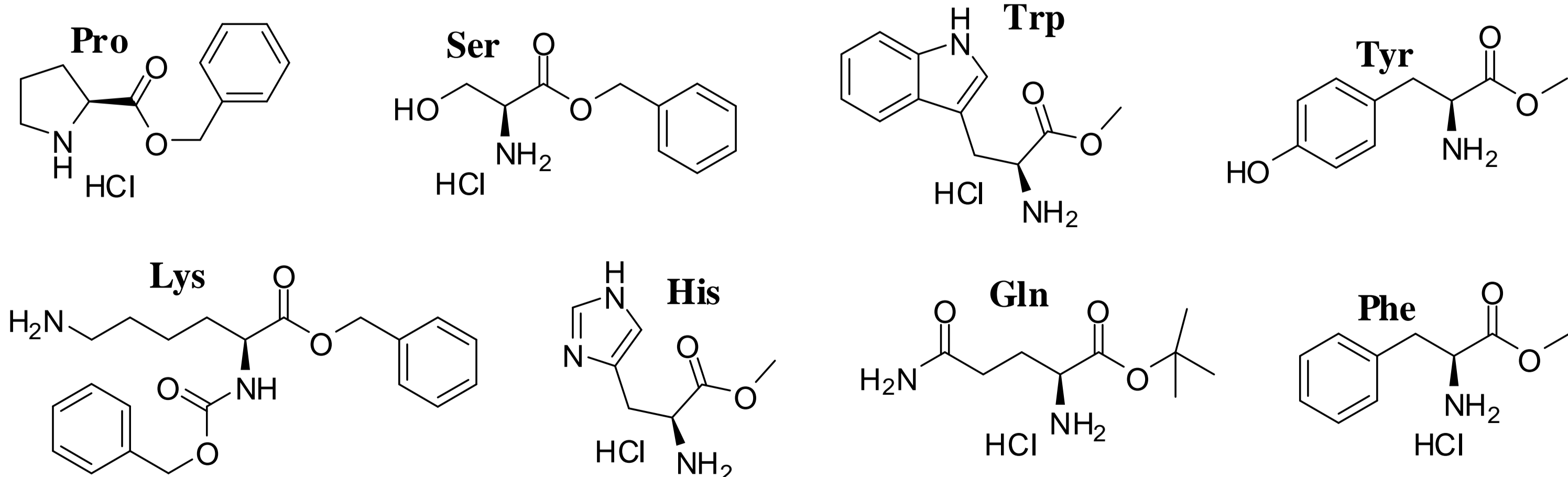
- Purification by cartridge (alumina – Al₂O₃)



Schematics of microfluidic system used

Conjugation reactions of [¹⁸F]ESF

- With model amino acids (carboxy-protected), insulin (injectable formulation) and BSA in various solvent systems, concentrations and reaction times
- 20 μL [¹⁸F]ESF + 80 μL substrates at room temperature
- Purification by passing through alumina cartridge



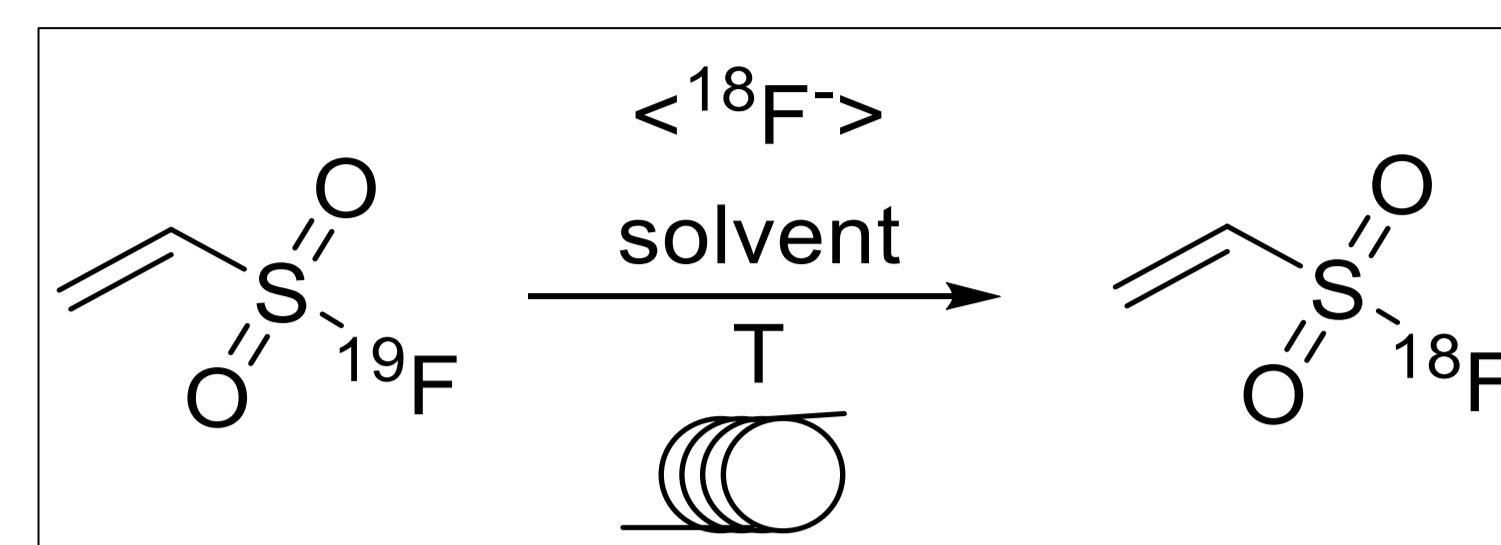
Structures of eight model amino acid analogues

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RESULTS

Radiosynthesis of [¹⁸F]ESF

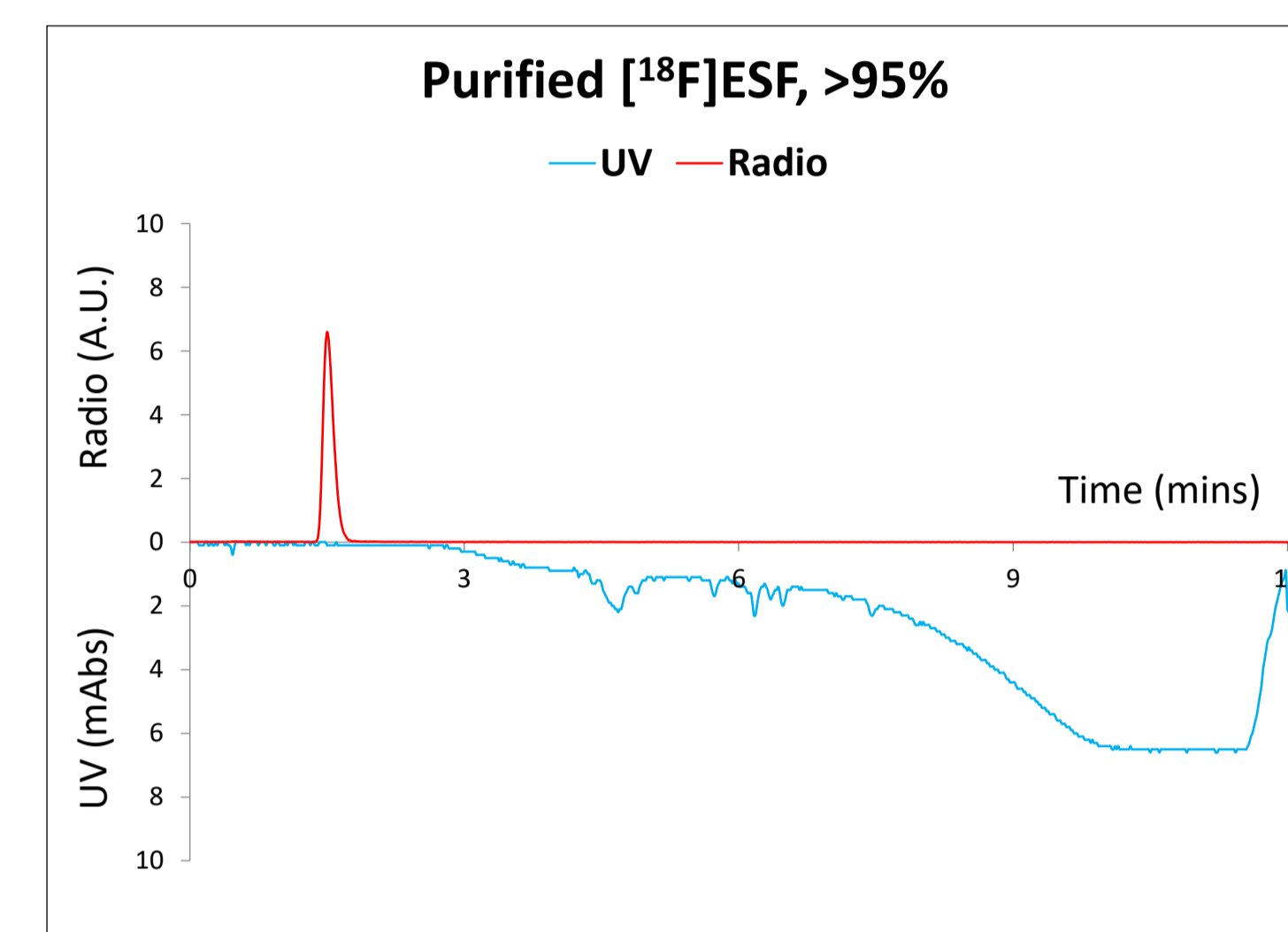
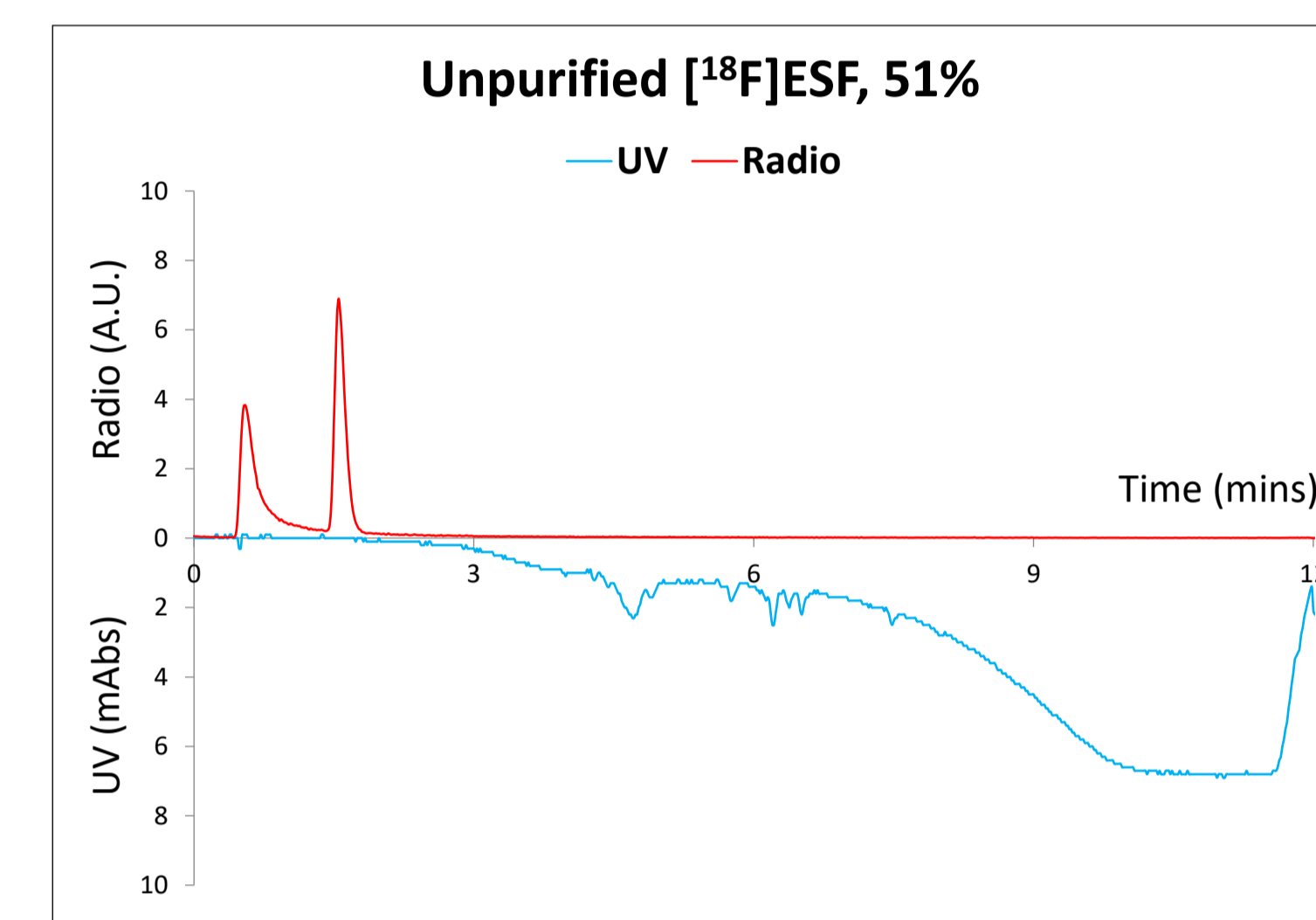


Ideal conditions:

- Activator: saline
- Solvent: water
- Temperature: 100 ° C
- Concentration: 5 mg/mL
- Residence time: 47 s (20 μL/min flow rate)

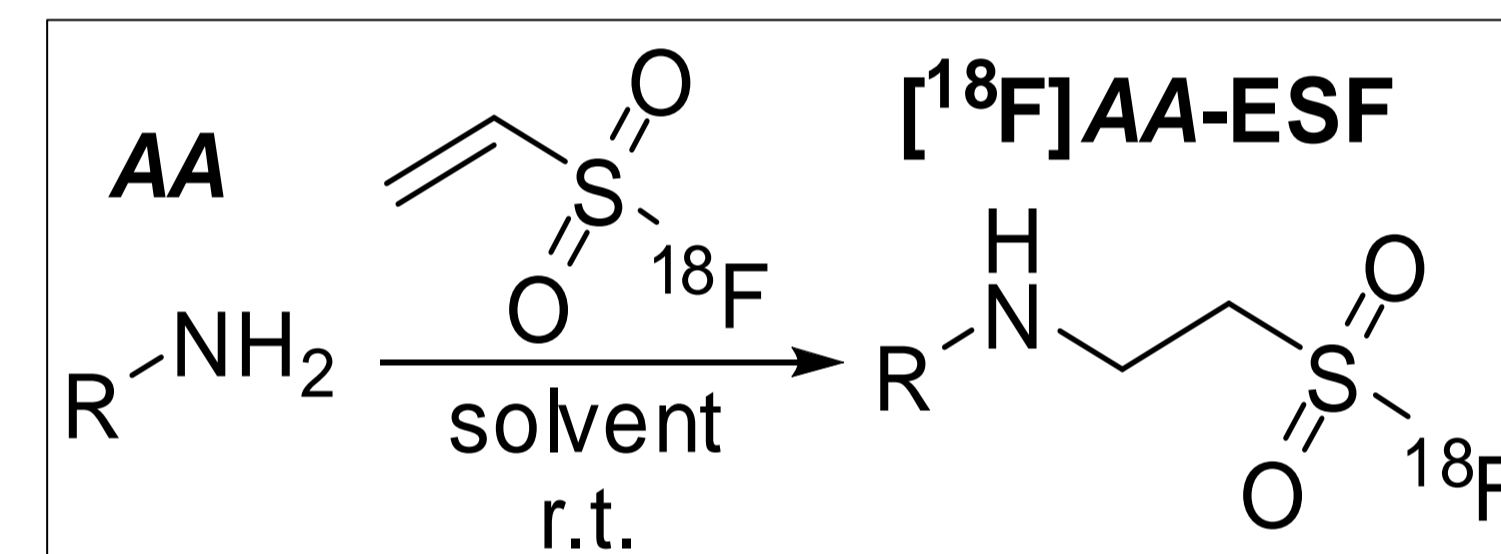
| | [ESF] [†] | [¹⁸ F]fluoride complex [*] | T (C) | Time (s) | RCY [‡] |
|----|----------------------|---|-------|----------|------------------|
| 1 | 20 (DMF) | K _{2.2.2} /K ₂ CO ₃ (CH ₃ CN) | 100 | 47 | 50% |
| 2 | 20 (DMF) | TEAB (CH ₃ CN) | 100 | 47 | 80% |
| 3 | 20 (DMF) | [¹⁸ O]H ₂ O | 100 | 47 | 32% |
| 4 | 20 (0.9% NaCl) | 0.9% NaCl | 100 | 47 | 90% |
| 5 | 5 (0.9% NaCl) | 0.9% NaCl | 100 | 47 | 81% |
| 6 | 2 (0.9% NaCl) | 0.9% NaCl | 100 | 47 | 33% |
| 7 | 5 (0.9% NaCl) | 0.9% NaCl | 130 | 47 | 46% |
| 8 | 5 (0.9% NaCl) | 0.9% NaCl | 70 | 47 | 50% |
| 9 | 5 (0.9% NaCl) | 0.9% NaCl | 100 | 94 | 72% |
| 10 | 5 (0.9% NaCl) | 0.9% NaCl | 100 | 19 | 73% |
| 11 | 5 (H ₂ O) | 0.45% NaCl | 130 | 47 | 64% |

[†]Concentration expressed in mg/mL, solvent in brackets; ^{*}No azeotropic drying, except entry 1; [‡]Assayed by RadioHPLC



HPLC profiles of unpurified and purified [¹⁸F]ESF

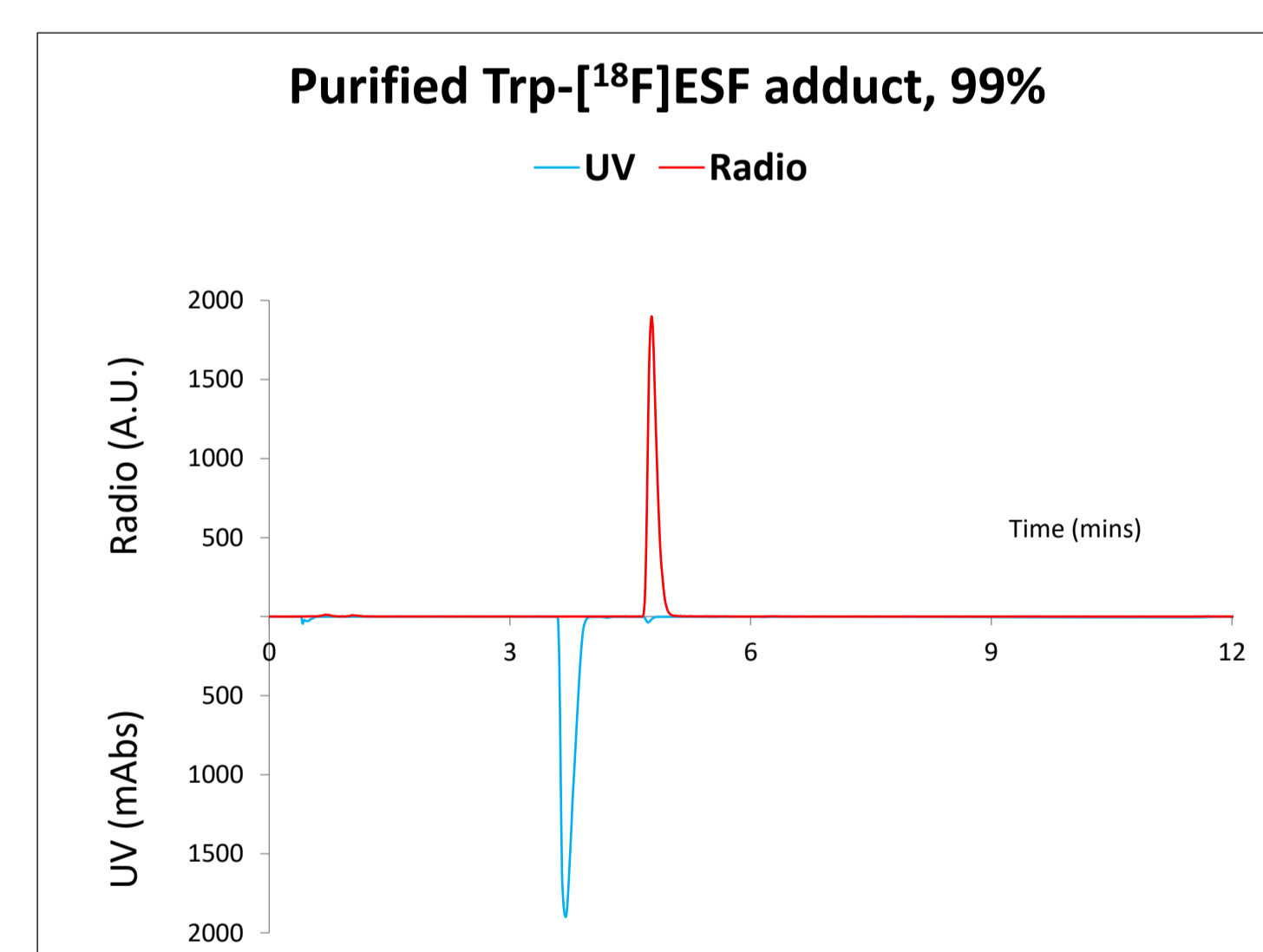
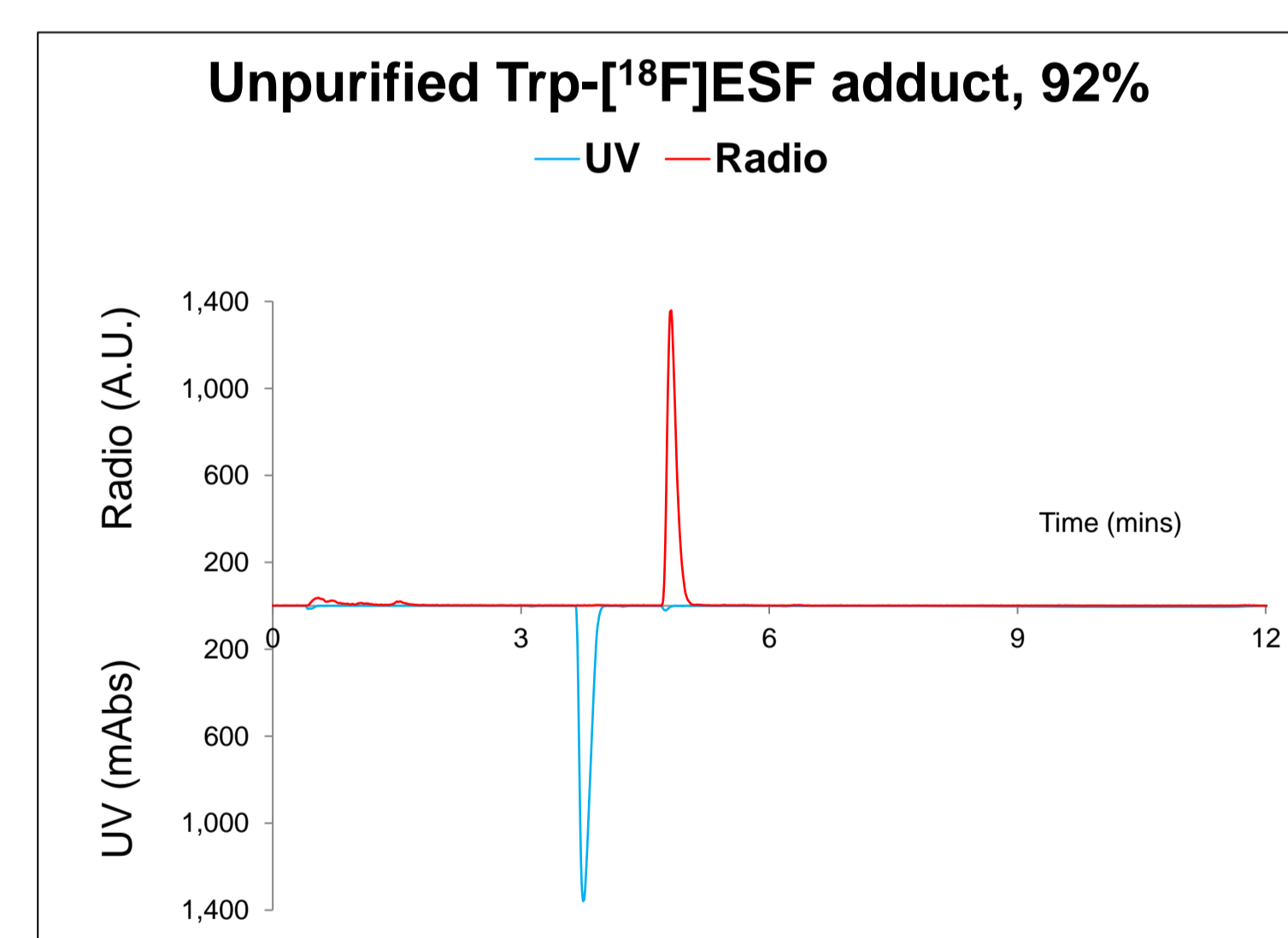
Conjugation reactions of [¹⁸F]ESF



- Model amino acids: RCY > 40% except His
- BSA: RCY 30%
- Injectable insulin: RCY 20%

| Precursors | RCY in different solvents | | |
|------------|---------------------------|------|---------|
| | DMF | DMSO | Ethanol |
| Ser | 72% | 68% | 70% |
| Phe | 52% | 53% | 61% |
| His | 0% | 0% | 0% |
| Trp | 48% | 41% | 70% |
| Pro | 77% | 71% | 83% |
| Gln | 51% | 42% | 55% |
| Tyr | 84% | 85% | 36% |

Radiochemical yield (RCY) of [¹⁸F]ESF conjugation



HPLC examples of unpurified and purified Trp-[¹⁸F]ESF adduct

CONCLUSION

Synthesis of [¹⁸F]ESF

- Straightforward reaction
- No HPLC purification needed

Conjugation reactions of [¹⁸F]ESF

- Successful with model amino acids (except His), insulin and BSA at room temperature
- No HPLC purification needed

| Prosthetic group | Radiolabeling | Easy synthesis | No target modification | Easy purification |
|-------------------|---------------|----------------|------------------------|-------------------|
| SFB related | ✓ | ✗ | ✓ | ✗ |
| Maleimide related | ✓ | ✗ | ✓ | ✗ |
| Click chemistry | ✓ | ✗ | ✗ | ✗ |
| SiFA | ✓ | ✓ | ✗ | ✓ |
| Boronates | ✓ | ✓ | ✗ | ✓ |
| AIF | ✓ | ✓ | ✗ | ✓ |
| ESF | ✓ | ✓ | ✓ | ✓ |

Comparison with other prosthetic group³⁻⁵

ACKNOWLEDGEMENT



MONASH University

